

# Walnut Pollination Dynamics: Pollen Flow in Walnut Orchards

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## Abstract

We have been conducting analyses of pollination dynamics in a California 'Chandler' walnut (*Juglans regia*) orchard. Our objectives are to document effective sources of pollen during the dichogamous bloom cycle of the two cultivars present in the orchard by determining pollen parentage of nuts, to describe the bloom phenology and to develop recommendations on pollinizer requirements in walnut orchards consistent with management of blackline disease and pollen-induced pistillate flower abortion (PFA). Trees are spaced at 28.5m. Prevailing winds are from the north and west. The orchard has 'Cisco' pollinizers every fourth row along the north and west outside rows of the orchard and there is a single 'S. Franquette' tree on the east edge. Other potential pollinizers are approximately a kilometer distant. We are analyzing pollen flow by conducting paternity analyses using microsatellite (SSR) DNA markers on nuts collected from trees at various distances from the pollinizer rows. Our results for 2001 are based on nuts harvested from flowers tagged at two bloom dates. The results show much higher than expected levels of pollen parentage originating from sources outside the orchard (i.e. neither self pollen, 'Cisco' pollen nor 'S. Franquette' pollen). During Chandler staminate full bloom, when copious amount of self pollen is present in the orchard, high levels of non-self paternity occur at the upwind portions of the orchard. For 2002, we expanded our level of temporal resolution by tagging flowers 8 times over the bloom cycle. SSR-based paternity analyses of approximately 900 nuts identified according to bloom date and orchard location again show high levels of paternity from sources outside the orchard. Because we cannot exclude 'Chandler' or 'Cisco' pollen from sources outside the orchard, it is likely that our estimates of pollen from distant external sources is significantly higher than the data would indicate.

## INTRODUCTION

Pollination requirements in walnut orchards are inadequately understood. Pollination relationships in this species are more complex than is typical of other orchard crops. The species is self compatible, however because it is also dichogamous, the period of overlap between pollen shed and pistillate flower receptivity may be inadequate for maximum productivity. Although there are no data on the relationship between pollen



load and walnut fruit set, we do know that excess causes pistillate flower abortion (PFA) (McGranahan, et al., 1994; Polito et al., 1996). In addition, CLRV-W, the viral agent that incites walnut blackline disease, is transmitted by pollen.

This uniquely complex pollination situation makes it essential that we know more about key parameters of walnut pollination. Unanswered questions address the basic reproductive biology of walnut as well as practical horticultural issues: How far does pollen travel in this wind-pollinated species? What are the limits of pollen load including the minimum necessary for fruit and nut set and the upper threshold that begins to trigger PFA? What is the role of the dichogamy cycle in pollen load? Is there a requirement for interplanting pollinizer cultivars to ensure adequate pollen load during the period when self pollen is not present? If, so how many pollinizers are adequate and what is the optimum orchard configuration to provide sufficient, but not excessive, pollen loads? To what extent does pollen from outside the orchard, present in any walnut growing area, contribute to fruit set?

Our objectives are to begin addressing these questions by determining patterns of pollen flow, viable pollen deposition on stigmas, and paternal inheritance in walnut orchards as they vary with distance from pollen source and with time during the dichogamous bloom cycle. We are using molecular genetic inheritance techniques to determine temporal and spatial patterns of pollen flow in an orchard.

## MATERIALS AND METHODS

We are working in a 'Chandler' orchard at 8.5m x 8.5m spacing. There are 'Cisco' pollinizers planted every fourth row along the north and west sides of the orchard. At the south edge of the orchard there is a single 'S. Franquette' tree in an adjacent 'Serr' orchard. East of the 'Serr' orchard is a 'Tehama' block. The nearest orchard with 'Chandler' or late-blooming pollinizer cultivars is approximately a kilometer distant. This presents a situation where the primary pollen sources in the orchard itself are well understood.

We conducted experiments in this location in 2001 and 2002. In 2001 we tagged flowers on the Chandler trees along transects 1, 2, 4, 8 and 16 tree rows moving south, away from the Cisco pollinizers and transects 1, 2, 4 and 8 tree rows north of the 'S. Franquette'. We tagged pistillate flowers at a pre-receptive stage on two dates: April 13, at which time 'Chandler' was in full staminate bloom and no catkins had opened on the 'Cisco' pollinizers or the 'S. Franquette' tree; and April 24, when 'Chandler' staminate bloom was 95% complete and 'Cisco' was in full staminate bloom and the earliest catkins had begun to shed pollen on the 'S. Franquette.'

In 2002 we selected eight separate limbs each on 'Chandler' trees 1, 2, 4 8 and 16 rows from 'Cisco' trees. Every three to four days throughout the bloom cycle newly emerged pistillate flowers and catkins were tagged. Every flower and catkin on each limb was tagged noting time of bloom emergence on the pistillate flowers and time of pollen shed for the catkins. Tagging dates were: 12, 16, 19, 22, 25, 29 April and 3, 9 May. Receptive pistillate flowers on adjacent flowers were collected to determine pollen load on each tagging date. PFA and total fruit drop was determined for the tagged flowers. Microsatellite (SSR) markers were used to determine pollen parentage of representative nuts.

## RESULTS AND DISCUSSION

In 2002 we analyzed male and female bloom phenology. Figs 1 and 2 show pollen shed phenology for Chandler and Cisco and the proportion of Chandler to Cisco pollen available in the orchard during the bloom period. Fig 3 shows the pattern of emergence of Chandler pistillate bloom in 2002. The reduced number of flowers opening between 14 and 19 April is correlated with low temperatures during this period (Fig. 4.).

Early flower drop, likely due to pollen-induced pistillate flower abortion (PFA), was high during the early bloom period (60% of the flowers that bloomed in that period), however; few flowers (<5% of total bloom) were open during that time. Subsequent PFA



was approximately 20% during the period of full staminate bloom, and then fell to approximately 10% (Fig. 5) as 'Chandler' staminate bloom ended (Fig. 5). Fruit set, throughout the bloom period, was negatively correlated to PFA ( $n = 8$ ,  $r = -0.89$ ,  $P = 0.003$ ). The PFA results are consistent with pollen load determinations (Fig. 7) and previous findings showing strong positive correlations between pollen load and PFA.

At harvest we determined fresh weight of nuts. These results are seen in Fig 8. Late blooming flowers produce significantly smaller nuts than early and mid bloom flowers.

The SSR markers we used to determine paternity were selected to differentiate the cultivars present in the orchard ('Chandler' and 'Cisco') and common in the area at the time of 'Chandler' bloom ('Franquette'). We also included 'Tehama' in our analyses because that is the cultivar grown in the adjacent orchard on the downwind side although staminate bloom in this cultivar is complete prior to 'Chandler' pistillate bloom.

Outcrossing analysis (Ritland, 1990) for 2001 (Fig. 9) indicates that for Chandler flowers tagged at the pre-receptive stage during full staminate bloom of Chandler (13 April) 8.5 m from the northern edge of the orchard, approximately 50% of nuts result from non-self crosses. This falls off to less than 20% one additional tree row distance (17 m) into the orchard and to 2-3% beyond that. Thus, despite the high levels of self pollen available in the orchard, there is a contribution at the edges greater than one might expect from bloom conditions in the orchard. For the second tagging period, 24 April, outcrossing rates exceeded 75% through 4 tree rows (34 m) and remained over 40% as far as 156 m from the pollinizer rows.

Results from 2002 are shown in Table 1. Several clear trends emerge from these data. Data for 12 April are not included. There were only 11 nuts and all had 'Chandler' as the pollen parent. By 16 April 'Cisco' parentage began to become apparent. Cisco's contribution to pollen parentage increased with time. However, there was a strong trend of reduced 'Cisco' parentage with distance from the pollinizer row. At distances from 34 m and greater, 'Cisco' parentage was similar to that of other sources including 'Franquette.' This indicates that the contribution of the pollinizers in the orchard have little effect beyond 4 to 8 orchard rows. A second trend is that the role of 'Chandler' as a pollen parent extends well beyond the time that would be predicted from the phenology of staminate bloom. From 25 April, when Chandler staminate bloom had dropped to very low levels relative to Cisco (Fig. 1) there was strong effect of Chandler pollen, especially at distances greater than 34 m from the Cisco pollinizers. Thus, while there is an important contribution from the Cisco pollinizers for trees within 17 m, the value of having these pollinizers appears minimal as there is sufficient self pollen and pollen from outside the orchard available to set fruit beyond the influence of the Cisco pollinizers.

These results suggest some tentative conclusions. One is that the contributions from individual trees do not appear to extend distances greater than approximately 30 m. In this range the contributions of pollen originating from the 'Cisco' pollinizers falls off to levels that do not appear to differ from that of cultivars not present in the subject orchard. It is reasonable to assume that some portion of the 'Cisco' parentage we find at these distances comes from sources outside the orchard as well, as 'Cisco' is a common pollinizer in 'Chandler' orchards in the area. Thus, the contribution from the orchard pollinizer trees is somewhat less than that indicated in the table 1. This conclusion suggests that in walnut-growing areas, large amounts of airborne pollen are naturally present.

What then is the significance of this conclusion for managing PFA and walnut blackline disease? Maintaining minimal losses to pollen-induced pistillate flower abortion is best achieved by minimizing pollen levels in the orchard to no more than that required for fruit set. The present results suggest that this can be done by removing pollinizers as their contribution to fruit set appears to be redundant to other, existing sources and they increase pollen loads in adjacent tree rows. Where blackline disease is prevalent, however, the fact that there is an influx of pollen from sources outside the orchard could lead to infection by blackline virus in orchards that are free of the virus. In these

situations there may be an advantage to regular spaced pollinizer rows to supply uninfected pollen at elevated levels sufficient to outcompete potentially infected pollen from outside the system.

#### **ACKNOWLEDGEMENTS**

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#### **Literature Cited**

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## Tables

Table 1. Paternity of 'Chandler' nuts inferred from SSR markers for bloom date of flower and distance from 'Cisco' pollinizer row. Analysis based on assumption of self ('Chandler'), 'Cisco,' 'S. Franquette' or 'Tehama' pollen parent.

Calendar Date	Julian Date	Dist (m)	Paternal Parent (Number of Nuts)					Other*
			Chand.	Cisco	Franq.	Tehama		
16-Apr	106	8.5	17					1
16-Apr	106	17	29	6				3
16-Apr	106	34	8	1	1			2
16-Apr	106	68	28	1	1			5
16-Apr	106	136	26	3				2
19-Apr	109	8.5	3	12				1
19-Apr	109	17	14	7	1	1		4
19-Apr	109	34	7	6				5
19-Apr	109	68	13	1				3
19-Apr	109	136	11	1	1			2
22-Apr	112	8.5	8	21	1	1		6
22-Apr	112	17	12	31				17
22-Apr	112	34	6	14	2			8
22-Apr	112	68	35	13	2			19
22-Apr	112	136	28	7				9
25-Apr	115	8.5	6	28	4			9
25-Apr	115	17	8	28	4			8
25-Apr	115	34	10	15	7			5
25-Apr	115	68	12	15	6			18
25-Apr	115	136	12	4	5			7
29-Apr	119	8.5	3	9				3
29-Apr	119	17		1				1
29-Apr	119	34	7	2	1			3
29-Apr	119	68	1	5	2			1
29-Apr	119	136	5					1
3-May	123	8.5	2	8	2			2
3-May	123	17		3	1			1
3-May	123	34		2				
3-May	123	68	2	5				1
3-May	123	136	8		5			
9-May	129	8.5	3	8	2			4
9-May	129	17		1				1
9-May	129	34	1	5	1			7
9-May	129	68	2	2				1
9-May	129	136	1		1			1

\*Nuts with multiple markers for outcrossing and genetic profiles inconsistent with Chandler, Cisco, Franquette or Tehama paternity.

## Figures

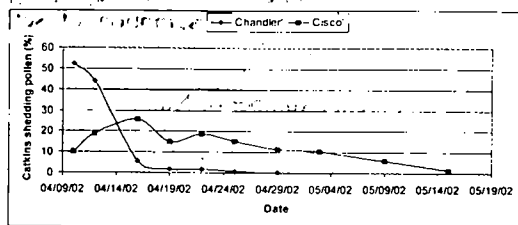


Fig. 1. Catkin shed by Chandler and Cisco during the 2002 bloom cycle.

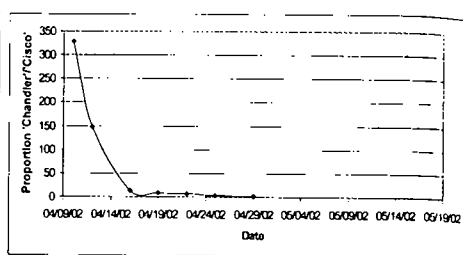


Fig. 2. Relative amounts of Chandler and Cisco pollen in the orchard during the 2002 bloom cycle.

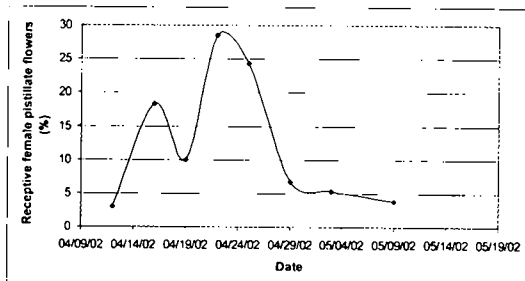


Fig. 3. Pattern of emergence of Chandler pistillate bloom in 2002.

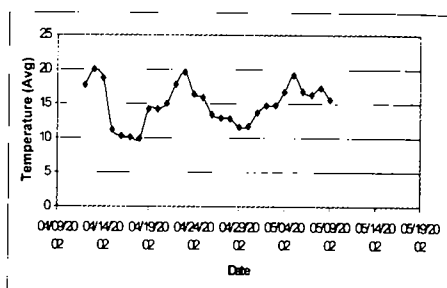


Fig. 4. Mean daily temperatures (C) for 2002 Chandler bloom period.

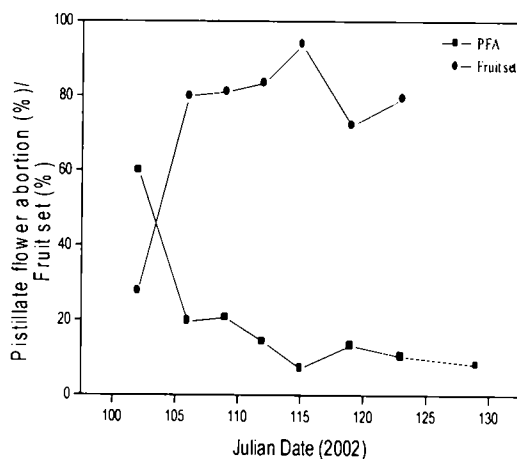


Fig. 5. Chandler PFA and fruit set in 2002 for flowers tagged throughout the bloom cycle.

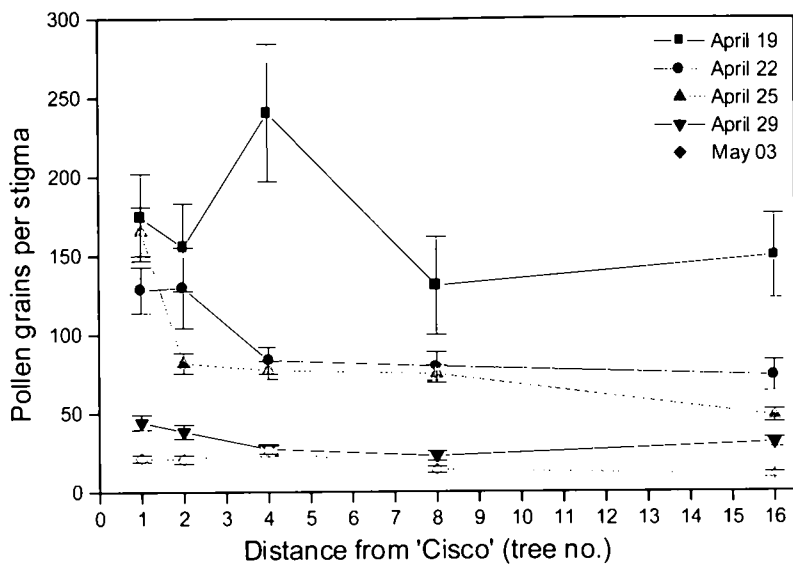


Fig. 6. Number of pollen grains per stigma during the Chandler bloom period and along distance transects from the Cisco pollinizers.

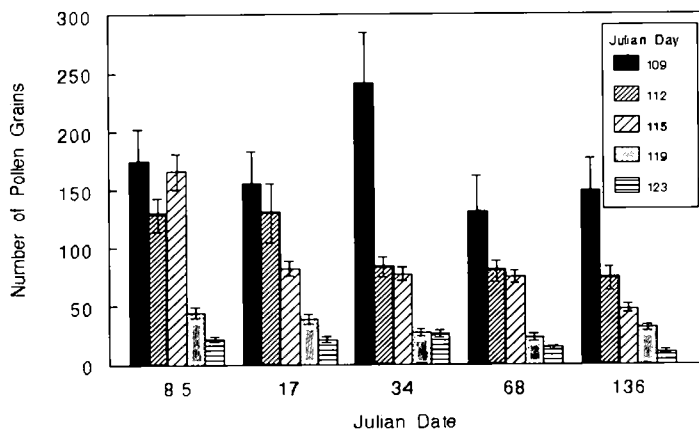


Fig. 7. Pollen load on 'Chandler' flowers.

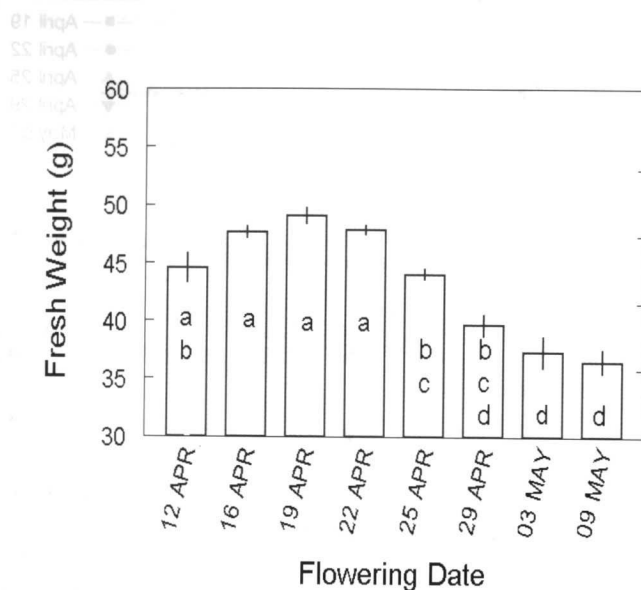


Fig. 8. Fresh weight of nuts developing from flowers from different bloom dates.

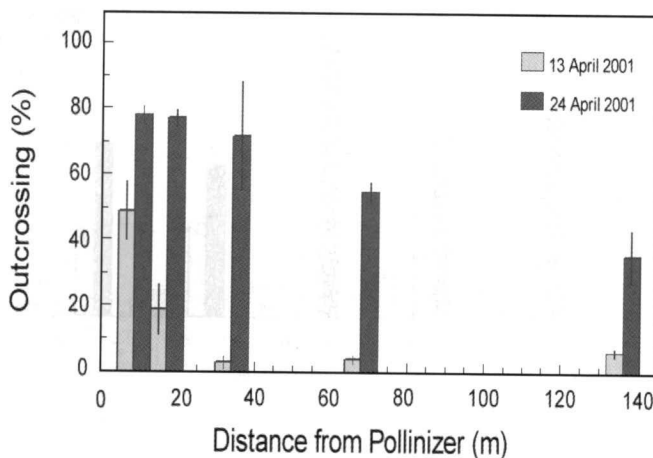


Fig. 9. Outcrossing rates for 'Chandler' trees with distance from 'Cisco' pollinizers (2001). Flowers were tagged at a pre-receptive stage prior to 'Cisco' staminate bloom (13 April) and after 'Chandler' staminate bloom (24). Ritland MLTR analysis.